Antifungals

Introduction

Antifungals take advantage of the unique properties of fungi, using them as targets of therapy. In particular, the **ergosterol** in the plasma membrane and the **glucans** in the cell wall are prime targets for antifungal therapy. But because fungi are eukaryotic, as our cells are, the drugs that work on fungi sometimes work on our cells, too. Fungi have cytochrome P450 enzymes in mitochondria. Ergosterol is a sterol, and has a structure similar to cholesterol. Fungi use DNA and RNA in a nucleus, just like our cells do. That means both that cellular targets are limited to where we differ, and that we can anticipate toxic side effects of antifungals when delivered systemically. When used topically, locally applied to the fungus, those side effects will not be felt.

For many years, amphotericin B was the only effective antifungal drug that could be administered systemically. Others were so toxic that they could not be administered systemically. Amphotericin B is still quite toxic, albeit also very effective. Survival of patients with AIDS, transplants, and cancer have greatly increased the incidence of symptomatic systemic fungal infections. Fungi do not normally cause symptomatic disease in immunocompetent individuals. The increased incidence of fungal disease and the lack of safe treatment provided impetus to develop newer antifungal agents.

The development of newer agents has allowed us to use less-toxic medications systemically. By getting less toxic, the drugs generally became more limited, capable of fighting specific fungi rather than being generally "antifungal." Amphotericin B remains the drug to fight all fungal infections, the default for severe illness or disseminated disease. What you will learn in this lesson is how to associate a fungus or a disease a fungus causes with a drug or drug class. As long as it is not life-threatening or disseminated, amphotericin B will not be the right answer and there will be a clearly correct antifungal (or class). The drug classes and their mechanisms of action are shown in Figure 2.1.

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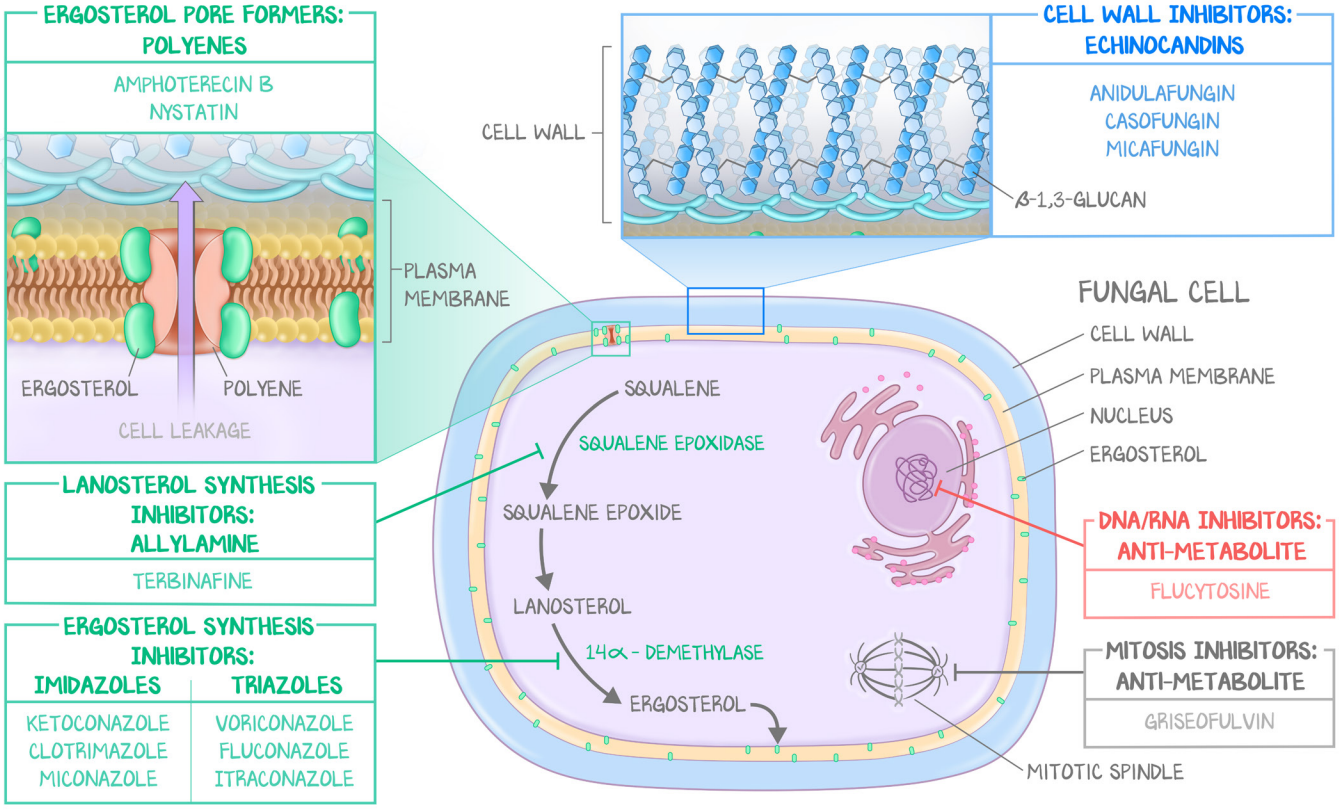

Figure 2.1: Mechanisms of Antifungals
Echinocandins target the cell wall, and have limited uses. Flucytosine is an antimetabolite and is used only for crypto meningitis in combination with amphotericin B. Griseofulvin is a mitosis inhibitor, but isn't the drug of choice for anything anymore. The vast majority of antifungals that are used target the fungal plasma membrane. And, in one way or another, that means ergosterol.

This lesson covers polyenes (ergosterol pore formers), imidazoles and triazoles (ergosterol synthesis inhibitors), benzylamines (lanosterol synthesis inhibitors), and echinocandins (cell wall inhibitors), then covers specific drugs such as flucytosine (antimetabolite) and griseofulvin (mitosis inhibitor).

Plasma Membranes: Pore Formers

The polyenes bind to **ergosterol within the plasma membrane** to **form a pore** in that membrane. This allows the flow of ions and water that results in cell lysis. There are two polyenes—amphotericin and nystatin.

Amphotericin B is the **intravenous** polyene that is used to treat disseminated, systemic, and severe fungal infections. It can be used to treat any fungal infection. It is the broadest-spectrum antifungal we use. It is the **default antifungal** and is used in **any life-threatening** or **disseminated** infection. It can cross the blood-brain barrier, so is the treatment of choice in **cryptococcus meningitis** (but always together with flucytosine). It fights the deadly bugs no other antifungal can, so is indicated in **mucormycosis**. It is effective against any fungus and can be used to treat any fungal disease. But it is not used to fight limited, benign, or superficial fungi because it is also toxic, the fungicidal benefit outweighed by the systemic toxicity.

Amphotericin B has been nicknamed "ampho-terrible" because of its toxicity. When infused, patients experience fevers, chills, and rigors. Phlebitis develops at the site of infusion. But the thing you must watch out for the most is **nephrotoxicity**. Amphotericin B binds to sterols in plasma membranes. It binds more

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readily to ergosterol of fungal membranes than it does to cholesterol of human membranes, but since they are both sterols, amphotericin CAN bind to cholesterol in human membranes, doing to our own cells what we want it to do to the fungus. Drugs administered systemically are distributed systemically, which means the drug sees all tissues. In tissues where the fungus isn't present, where there is no ergosterol, the only target for the drug is cholesterol. That's why the patients feel so terrible when they receive the drug. The kidneys get it worst of all. Irreversible tubular necrosis occurs because of amphotericin's effect on the distal tubule. A reversible, prerenal component can be improved by infusing normal saline pre- and post-infusions. The irreversible tubular necrosis is inevitable except with improved formulations.

Newer **liposomal formulations** package the active amphotericin B in lipid vehicles. Amphotericin B's affinity for the lipid vehicle lies somewhere between its affinity for ergosterol and its affinity for cholesterol. The net effect of that affinity gradient is that amphotericin leaves the vehicle only when near ergosterol (higher affinity for ergosterol than the lipid vehicle) but remains bound to the vehicle when near only cholesterol (lower affinity for cholesterol than the lipid vehicle). The vehicle acts both to protect the human cells from amphotericin B activity and as a reservoir to act on fungal cells.

Nystatin is the other polyene. It cannot be administered systemically because it has an even greater toxicity than amphotericin. It can be used topically to treat some skin infections, though the convenience of the azoles' administration usually supplants nystatin. Nystatin should be learned in the context of treating mucosal candidiasis. Because it does not cross the blood-gut barrier, oral ingestion does not equate with systemic administration, and is effectively a "topical" treatment of the mucosa. It is used to treat oral candidiasis (swish and spit) and to treat esophageal candidiasis (swish and swallow).

Plasma Membranes, Ergosterol Synthesis Inhibitors

The **azoles** are a class of medications that inhibit the CYP450-dependent demethylation of lanosterol, mediated by 14-α-demethylase, one of the final steps in synthesizing ergosterol. Inhibiting this step, therefore, **inhibits ergosterol synthesis**. Because ergosterol is part of the plasma membrane, these drugs **inhibit cell membrane synthesis**. All azoles inhibit CYP450 enzymes in fungi. All azoles also inhibit CYP450 in humans. CYP450 enzymes are in the liver. All azoles therefore have the side effect profile as being **CYP450-inhibitors** (and so can affect metabolism of other drugs) and causing **hepatotoxicity**. All azoles also inhibit human 17,20-desmolase and 17-α-hydroxylase (these enzymes you should not recognize immediately; but know that they are involved in androgen synthesis), and therefore **inhibit androgen synthesis**, resulting in **gynecomastia**.

All azoles can be divided into two types—imidazoles and triazoles.

The **imidazoles** are toxic when administered systemically and can only be used topically. These are ketoconazole, clotrimazole, and miconazole. They come in the form of creams, powders, and aerosols. These are used for skin infections, treating the dermatophytes (tinea pedis, cruris, corporis, etc.). Imidazoles are ineffective at treating fungal infections that have penetrated the nails or hair—those require systemic antifungals and imidazoles cannot be used systemically. Clotrimazole comes as a troche (a lozenge) and can be used to treat oral candidiasis, though fluconazole and nystatin are more often used. Learn "*imidazoles are topical treatments for dermatophyte infections*."

The **triazoles** are less toxic and so can be administered systemically. There are three triazoles that we want you to tether to three diseases/species—fluconazole with *Candida*, voriconazole with *Aspergillus*, and itraconazole with limited dimorphic fungi. **Fluconazole** can be administered orally or intravenously. It is used to treat *Candida* infections of all types, but is commonly seen in the treatment of AIDS-related candida esophagitis. Fluconazole is also used in the maintenance phase of cryptococcal meningitis (after induction with amphotericin B and flucytosine). A single dose of fluconazole can treat vulvovaginitis caused by *Candida*. **Voriconazole** is used for Aspergillus **infections** (ABPA, aspergilloma,

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and invasive aspergillosis). **Itraconazole** is used to treat pulmonary disease caused by the dimorphic fungi *Histoplasma*, *Blastomycoses*, and *Coccidioides*. However, if these fungi disseminate, amphotericin B must be used instead.

Plasma Membranes, Ergosterol Synthesis Inhibitors

The allylamines and the benzylamines (the "-ylamines") inhibit an early step in lanosterol synthesis, **squalene epoxidase**. If lanosterol is not synthesized, there is no substrate for ergosterol synthesis, so these drugs also inhibit ergosterol synthesis, so also **inhibit cell membrane synthesis.** There are several drugs in this class, but only one you should be familiar with—the allylamine *terbinafine*.

Terbinafine can be used as a topical treatment for **dermatophyte infections** interchangeably with the imidazoles. There is growing literature on the treatment of dermatophyte infections that suggests terbinafine is superior (faster cure rates reducing treatment duration) to the imidazoles for treating dermatophyte infections. However, treating superficial dermatophyte infections involves topical treatments, the imidazoles are available over the counter, and the decision to choose one over another is usually up to patient preference and cost. Terbinafine comes only as a cream and is more expensive. The imidazoles come as creams, powders, and aerosols. Moreover, keeping the skin dry and clean has just as much impact as treating it with medications. You should not be asked to choose between imidazoles and terbinafine for superficial dermatophyte infections.

Terbinafine is **clearly the right answer** in one instance. Dermatophyte infections of the nail (**onychomycosis**) will not respond to topical therapy. Therefore, systemic therapy must be chosen. Since the imidazoles cannot be administered systemically, **terbinafine** is the only choice.

In addition, until the creation of terbinafine, the only oral option available for onychomycosis was griseofulvin, discussed at the end of this lesson. Oral terbinafine has replaced griseofulvin as the drug of choice to treat fungal nail infections and has rendered griseofulvin obsolete.

Even though terbinafine is not at all associated with P450 enzymes, systemic terbinafine, more than the azoles, causes **hepatotoxicity**. Patients should be counseled to avoid alcohol during the treatment duration for onychomycosis, which is generally around 90 days. As topical treatments are not absorbed through the skin, the only concern for hepatotoxicity is in the oral formulation.

Cell Wall Inhibitors: Echinocandins

Fungal cell walls are made of chitin and β-glucans. Chitins and β-glucans reinforce one another to maintain the integrity and strength of the fungal cell wall. Compromise one, you compromise the entire cell wall. Echinocandins target **glucan synthesis**. The specific target is **β-1,3-glucan synthase**. β-1,3-glucan synthase is a plasma membrane enzyme that assembles and delivers 1,3-β-glucan into the periplasmic space outside the fungal cell. The effect is that echinocandins are fungal **cell wall inhibitors**.

The echinocandins are particularly useful in fighting *Candida albicans* infections. They are intravenous only, so are used for severe or disseminated disease. The echinocandins are THE best example of trading spectrum for side effects. The echinocandins are very well tolerated, far more than amphotericin B. And yet the echinocandins are almost never the empiric antifungal of choice because their spectrum is so narrow. **Candidemia** (caspofungin, micafungin) and **neutropenic fevers** (caspofungin) are the only real indications for echinocandins. A neutropenic fever is defined by a temperature greater than 38°C (100.4°F) while the absolute neutrophil count is below 500.

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Where echinocandins shine is after the fungal culture reveals susceptibility to echinocandins. This is not going to be how you interact with echinocandins in the basic sciences. When dealing with an actual patient, you may see them at admission, put them on amphotericin B for invasive fungus, then receive the fungal culture results days later. For the basic sciences, equate echinocandins and invasive *Candida albicans*.

DNA/RNA Inhibitors: Flucytosine

Flucytosine is delivered as a prodrug. It is taken into fungal cells through transporters not present in human cells. In the fungus, it is converted to 5-fluorouracil (5-FU) by cytosine deaminase. Flucytosine is a uracil analog. Flucytosine gets made into a nucleotide that looks like uracil (5-FU). Uracil is normally used to make thymidine. **Thymidylate synthase** uses uracil as a substrate and adds a methyl group to the 5 position. But when thymidylate synthetase tries that on 5-FU, there's already a fluorine there on the 5 position. Thymidylate synthetase fails. Thymidylate synthetase tries again. Whoopsie, a fluorine. In effect, 5-FU **inhibits thymidylate synthetase**, thereby inhibiting thymidine synthesis, ultimately disrupting transcription (proteins) and replication (division).

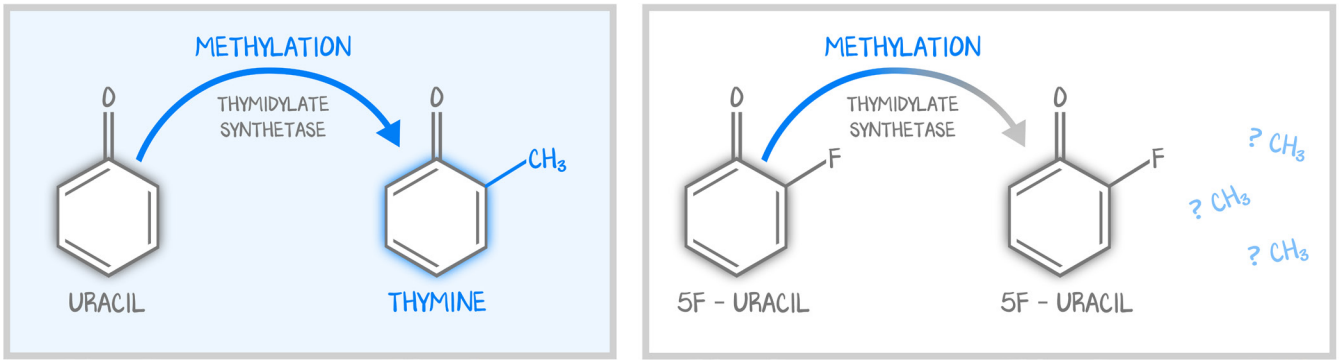

Figure 2.2: Flucytosine Mechanism of Action
The fluorine at the 5 position prevents the formation of thymidine from uracil.

Flucytosine is taken into fungal cells by a transporter human cells don't have. Flucytosine is converted into 5-FU at a much faster rate in fungal cells than it would in human cells if it did get into human cells. But the laws of thermodynamics don't allow for absolutes.

You may recognize **5-fluorouracil** as a chemotherapeutic agent. It was classified as an antimetabolite, specific to the synthesis phase of cell division. It is known for causing bone marrow suppression. Knowing that it will "only work in the fungus," we administer very high levels of flucytosine. With such high levels of flucytosine, it is possible that some of that flucytosine ends up as 5-FU in human cells. Even a small amount can cause problems for cells that are rapidly dividing or are very transcriptionally active, such as the bone marrow. An anticipated, but uncommon side effect of flucytosine is **bone marrow suppression.**

Its only use is in the treatment of cryptococcal meningitis, which is treated with a combination of amphotericin B and flucytosine. Never use flucytosine as monotherapy, as resistance develops quickly.

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Mitosis Inhibitors

There is only one medication in this category, **griseofulvin**. It inhibits microtubule formation; consequently, it **disrupts the mitotic spindle**, and **inhibits mitosis**. It is an alternative to terbinafine for hair and nail dermatophyte infections. Before terbinafine existed, griseofulvin was the drug of choice. Now it remains on the pharmacology list and plagues learners with its incomprehensible name because of its mechanism of action. Its mechanism of action in fungus resembles the mechanism of action of paclitaxel in humans. It binds to and inhibits disassembly of the mitotic spindle, allowing cytokinesis to occur, but preventing the tubulin dimers from being able to reform the cytoskeletal microtubules.

MEBENDAZOLE	GRISEOFULVIN	COLCHICINE	VINCRISTINE	PACLITAXEL
Helminths	Fungi	Inflammation	Cancer	Cancer

Table 2.1: Clinical Correlations
Other drugs that act on microtubules.

We don't use griseofulvin anymore because it is such a dirty drug. It is **teratogenic**, causes **disulfiram-like reactions** (emesis with alcohol), it **induces cytochrome P450** enzymes responsible for **warfarin metabolism**, and has been shown to be carcinogenic. It is a mitosis inhibitor, so is fungistatic. It is delivered into the keratinocyte precursors, and those cells must grow out towards the epithelium to take effect. For both of these reasons, treatment duration was for up to a year. Long duration and toxic side effects make it a drug not worth using anymore.

	CLASS	EXAMPLE	MECHANISM	NOTES
Cell wall stress	Echinocandins	Micafungin Caspofungin	Inhibits cell wall synthesis by inhibiting β-1,3-glucan synthase	Aspergillosis and refractory candida
DNA and RNA stress	Antimetabolite	Flucytosine	Converted into 5-FU which inhibits DNA and RNA synthesis through chain-length termination and inhibition of thymidylate synthase	Used with amphotericin B only. Targets DNA, so human cells vulnerable—bone marrow suppression
Cell membrane stress	Imid-azoles	Ketoconazole Clotrimazole Miconazole	Inhibits ergosterol synthesis by inhibition P450 enzyme 14 α-sterol demethylase	(Toxic, so topical), P450 inhibitors in humans
	Tri-azole	Fluconazole Voriconazole Itraconazole		(Less toxic, so systemic), P450 inhibitors in humans
	Allylamines and benzylamines	Terbinafine (A) Naftifine (B) Butenafine (B)	Inhibits ergosterol synthesis by inhibition of squalene epoxide	Terbinafine topically for tinea, terbinafine orally for onychomycosis
	Polyenes	Amphotericin B Nystatin	Forms pores in plasma membranes	Amphotericin B liposomal, slow infusion, renal failure
Microtubules	M phase inhibitor	Griseofulvin	Inhibits mitosis by preventing microtubule assembly	No primary indication,

Table 2.2: Drugs by Fungal Target

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USED TO TREAT SYSTEMIC INFECTIONS				
Polyenes	Echinocandins	Tri-azoles	Allylamines	M phase inhibitor
Amphotericin B (IV only)	Micafungin Caspofungin (IV only)	Fluconazole Voriconazole Itraconazole	N/A	N/A
USED TO TREAT SUPERFICIAL MUCOSAL AND SKIN INFECTIONS				
Polyenes	Echinocandins	Imid-azoles	Allylamines	M phase inhibitor
Nystatin (swish and spit)	N/A	Clotrimazole Ketoconazole Miconazole (creams and sprays)	Terbinafine (orally ingested for nail infection)	Griseofulvin (orally ingested for nail infection)

Table 2.3: Fungal Medications Organized by Severity of Infection

QUICK REACTION TREATMENTS			
INFECTION	**DRUG**	**INFECTION**	**DRUG**
Crypto meningitis	Ampho B + flucytosine	Mucormycosis	Amphotericin B
Candida in mouth	Nystatin swish and spit or Clotrimazole troches	*Candida* in blood	Micafungin
Candida in esophagus	Nystatin swish and swallow or Fluconazole	*Candida* in vagina	Fluconazole (PO) or Clotrimazole (PV)
Aspergilloma	Voriconazole	Invasive *Aspergillus*	Voriconazole
Local dimorphic fungi	Itraconazole	Dermatophytes without nail/hair, aka athlete's foot, jock itch, ringworm	Clotrimazole (top) Ketoconazole (top)
Disseminated dimorphic fungi	Amphotericin B	Dermatophytes with nail or hair involvement	Terbinafine (PO) Griseofulvin (PO)

Table 2.4: Fungus to Drug

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DRUG	USED FOR
Fluconazole	*Candida*
Voriconazole	*Aspergillus*
Itraconazole	Local Dimorphic fungi
Clotrimazole	Dermatophytes
Ketoconazole	Dermatophytes
Miconazole	Dermatophytes
Terbinafine	Nail fungus
Amphotericin	Disseminated dimorphic fungi, mucormycosis, cryptococcal meningitis, any life-threatening fungal infection

DRUG	USED FOR
Nystatin, spit	*Candida* mouth
Nystatin, swallow	*Candida* esophagus
Micafungin	Candidemia
Caspofungin	Neutropenic fevers
Potassium iodide	*Sporothrix*
Flucytosine	With amphotericin for crypto meningitis
Griseofulvin	Nothing

Table 2.5: Drug to Fungus

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